

ANALYSIS OF SPARFLOXACIN AND ITS DEGRADATION PRODUCTS BY BIOASSAY

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The *in vitro* activities of sparfloxacin and sparfloxacin after exposure to UV-light were analysed by microbiological assay. However, the low activity achieved with sparfloxacin exposed to UV-light was a source of concern and requires further investigation for its photodegradation mechanism.

Keywords: Bioassay; Fluoroquinolone; Photodegradation; Quality Control; Sparfloxacin; Drugstability

Introduction

Sparfloxacin (Fig. 1) a quinolone carboxylic acid derivative, is one of the recent fluorinated quinolones, which is active as an antimicrobial agent against a wide range of gram-positive and gram-negative organisms including mycobacteria (1, 2). A drawback of fluoroquinolone products is their photoreactivity (3-5). Sparfloxacin has been studied in terms of therapeutic activities (1, 2) however, few reports about its physicochemical analysis are available in the literature (6-8).

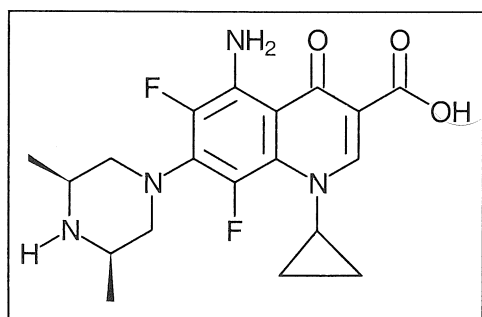


Fig. 1. Chemical structure of sparfloxacin

Due to the photosensitivity of sparfloxacin (3), the aim of this work was to develop an easy, rapid and sensitive method to determine the presence of any photodegradation product in the powder form. An accelerated study of stability in aqueous solution was carried out by subjecting a solution of sparfloxacin to UV-light (peak wavelength 290 nm) for five hours at room temperature. Sparfloxacin powder (99.5%) was supplied by Dainippon Pharmaceutical Co., Osaka, Japan and Rhone-Poulenc Rorer, USA. All other chemicals used were of analytical grade. For the determination of biological activity a microbiological plate assay was performed.

Bacterial strain, media and growth conditions: The indicator organism was *Escherichia coli* NCTC 10418 grown in nutrient broth (Unipath, Basingstoke, UK) at 37°C overnight diluted in nutrient

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broth 1:200 (100 μ L overnight broth into 20 mL). Sterile Isosensitest agar (150 mL) was poured into a microbiological assay plate (243 x 243 x 18 mm) and stored at 4°C overnight. The diluted broth (20 mL) was inoculated to the surface of each plate. A 8 mm diameter cork borer was used to cut 25 wells into the agar, and 150 μ L of each sample was added to each well. Each sample was assayed in triplicate. The bioassay plates were incubated at 37°C aerobically for 18 h. The zone sizes were carefully measured with calipers. A standard curve was prepared by measuring the zone diameters of five concentrations (10 to 1000 $\text{mg}\cdot\text{L}^{-1}$) of sparfloxacin in pH 7.0 sodium phosphate buffer.

To photodegrade sparfloxacin, a fresh solution (1 $\text{mg}\cdot\text{L}^{-1}$) was submitted to UV light (290 nm) for 24 and 36 hours in a chamber (16 x 16 x 100 cm).

Sparfloxacin Tablets (200 mg): Ten tablets were ground up and five times the average weight were transferred to prepare 1000 $\text{mg}\cdot\text{L}^{-1}$ solution. This solution was placed in petri dishes in the UV light chamber for 24 hours.

The applicability of the proposed method for the determination of sparfloxacin and its degradation products was demonstrated by analysing six aliquots of the reference substance. The isolation of these photoproducts will be performed by preparative HPLC and their chemical structures determined by NMR, MS, UV and IR spectra.

The microbiological analyses were carried out to determine whether UV exposure gave rise to a loss of antibactericidal activity. After exposure of sparfloxacin solution or solution made from tablets, a significant loss of activity was observed (Fig. 2). After 24 hours exposure to UV, 30% sparfloxacin remained, and after 36 hours exposure this further decreased to 15%. After 24 hours

exposure to UV, the tablet solution retained 27 % activity. The results showed that the substance studied was sensitive to photodegradation. A large decrease in the concentration of sparfloxacin after exposure to UV-light was observed and detected by bioassay. This decrease was an important source of concern and suggests further studies about its photodegradation mechanism. The existence of photoproducts can induce side effects and toxicity as well as loss of activity aimed for treatment.

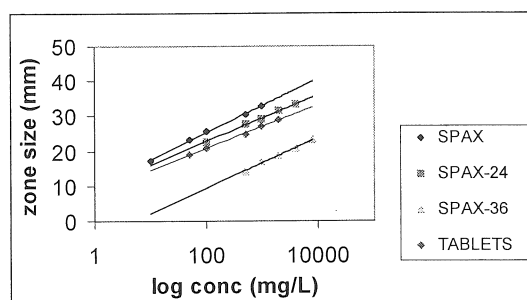


Fig. 2. Zone size mean for *E. coli* NCTC 10418 by sparfloxacin (SPAX) standard, tablets and sparfloxacin photodegrade solutions. (24 and 36 represent exposure hrs to UV.)

Acknowledgments

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References

- Goa, K. L., Bryson, H. M., Markham, A.: *Drugs* 53, 700 (1997)
- Shimada, J., Nogita, J., Ishibashi, Y.: *Clinical Pharmacokinetics* 25, 358 (1993)
- Engler, M., Rusing, G., Sorgel, F., Holzgrabe, U.: *Antimicrobial Agents and Chemotherapy* 42, 1151 (1998)

4. Marutani, K., Matsumoto, M., Otabe, Y., Nagamuta, M., Tanaka, K., Miyoshi, A. *et al.*: *Ibid.* 37, 2217 (1993)
5. Phillips, G., Johnson, B. F., Ferguson, J.: *J.Antimicrobial Chemotherapy* 26, 783 (1990)
6. Marona, H. R. N., Schapoval, E. E. S.: *J.Pharmaceutical and Biomedical Analysis* 20, 413 (1999)
7. Marona, H. R. N., Schapoval, E. E. S.: *J.Antimicrobial Chemotherapy* 44, 136 (1999)
8. Marona, H. R. N., Schapoval, E. E. S.: *Información Tecnológica* 9, 251 (1998)

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